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Establishment of a Rice Mutant Library for Functional Genomics

Yue-Ie Hsing[1a], Chyr-Guan Chen[2a], Ming-Jen Fan[3a], Sheng-Chung Huang[2], Po-Chang Lu[1], Ku-Ting Chen[4], Shuen-Fang Lo[4,5], Peng-Kai Sun[4], Shin-Lon Ho[6], Kuo-Wei Lee[4], Yi-Chieh Wang[4], Wen-Lii Huang[7], Swee-Suak Ko[8], Shu Chen[2], Jyh-Long Chen[4], Chun-I Chung[1], Yao-Cheng Lin[1], Ai-Ling Hour[1], Yet-Walt Wang[4], Ya-Chi Chang[4], Min-Wei Tsai[4], Yi-Show Lin[4], Yin-Chin Chen[4], Hsing-Mu Yen[2], Charng-Pei Li[2], Chiu-Kai Wey[2], Ching-Shan Tseng[2], Ming-Hsing Lai[2], Liang-Jwu Chen[5] and Su-May Yu[4]*

1 Institute of Plant and Microbial Biology, Academia Sinica, Taipei 115, Taiwan.
2 Taiwan Agricultural Research Institute, Wufong, Taichung 413, Taiwan.
3 Department of Biotechnology & Bioinformatics, Asia University, Lioufeng Rd., Wufeng, Taichung 413, Taiwan.
4 Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan.
5 Institute of Molecular Biology, National Chung-Hsing University, Taichung 400, Taiwan.
6 Department of Agronomy, National Chia-Yi University, Cha-I 600, Taiwan.
7 Department of Biotechnology, Fooyin University, Kaohsiung Hsien 831, Taiwan.
8 Academia Sinica Biotechnology Experimental Center in Southern Taiwan, Southern Taiwan Science Park, Tainan 741, Taiwan.

[a] Equal contributors

Corresponding author

Su-May Yu

Institute of Molecular Biology, Academia Sinica, Nankang,
Taipei 115, Taiwan, R.O.C.
Phone: 886-2-2788-2695
Fax: 886-2-2788-2695 or 886-2-2782-6085
E-mail: sumay@imb.sinica.edu.tw

ABSTRACT

Rice has been a model monocot for functional genomics research due to its small genome size (430 Mb) relative to other cereals, its ease of transformation, and its economic importance. With the completion of genomic sequencing analysis, the next challenge will be to assign functions of 37,000 predicted rice genes. During years 2002 to 2006, we have generated nearly 60,000 gene knockout or activation transgenic rice mutant lines using a T-DNA insertional mutagenesis approach. These mutant lines provide important resources for functional analysis of cereal genes using both forward and reverse genetics approaches. The forward genetics approach would screen for mutants with abnormal phenotypes grown under various conditions or treatments, and the reverse genetics approach would identify mutants based on information of flanking sequence tag (FST). By applying these approaches, currently, we have identified many mutants with altered phenotypes of important agronomic traits. Functions of genes responsible for these phenotypes are being analyzed. Novel genes with essential functions will be employed for cereal crop improvement through genetic engineering approaches.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important crops in the world. Rice, wheat, and maize together account for 60% of the world's food production, and rice itself is the principal food of nearly 50% of the world's population. These cereal crops share a large degree of synteny, making rice an excellent model cereal crop for genomics research (Gale and Devos, 1998). Rice was the obvious choice for the first genome sequencing of a crop plant for the following reasons: 1) Rice has the smallest genome

size (400 Mb) among the major cereal crops. 2) Rice can be transformed on a large scale on a routine basis. 3) Isolation of genes from rice could facilitate isolation of homologous genes from other cereal crops. 4) Much molecular and genetic information (ESTs, markers, genetic and physical maps, etc.) about rice is available.

With the completion of rice genomic sequencing, the challenge of the post-genome era is to understand the functions of the huge number of genes predicted by sequence information. To facilitate such an effort, several approaches have been developed. An important and direct approach of defining the function of a novel gene is to alter or eliminate its function by insertional mutagenesis. Insertional mutagenesis, using T-DNA or a transposable element, has the advantage that the inserted element acts as a tag for gene identification. Gene knockout by insertion of *Agrobacterium tumefaciens* derived transfer DNA (T-DNA) has been developed for tagging genes in various plant species. The advantages of using T-DNA tags are that T-DNA insertion is a random event, and that the inserted genes are stable through multiple generations (Azpiroz-Leehan and Feldmann, 1997). Additionally, T-DNA is usually inserted at low copy number in the plant genome, e.g., 1.5 copies per diploid genome in Arabidopsis and rice (Feldmann, 1991; Jeon *et al.*, 2000), which facilitates establishment of linkage between T-DNA-inserted gene and mutant phenotype.

T-DNA insertional mutagenesis has been successfully used to obtain loss-of-function mutants and identify genes from Arabidopsis (Azpiroz-Leehan and Feldmann, 1997; Krysan *et al.*, 1999) and rice (Jeon *et al.*, 2000; Jung *et al.*, 2003). However, this gene knockout tagging approach has some limitations, i.e., difficulty in identifying the function of redundant genes, or of genes required in early development. An alternative approach, gene activation tagging, has been designed to obtain dominant

mutants by activating genes adjacent to enhancers integrated into the genomic DNA through transformation. Advantageous features of the gene knockout/activation tagging approach for functional genomics include: 1) disruption of gene function leading to loss-of-function mutations, 2) activation of gene expression causing gain-of-function mutations, 3) the technique being a direct way to determine the function of a gene product *in situ*, and 4) the inserted marker being available for subsequent identification of disrupted genes.

Development of a large population of insertional mutant rice lines will be extremely valuable for functional analysis of rice genes. While these lines are important resources for forward genetic studies of gene function, their importance for reverse genetic studies also increases with the availability of rice genome sequence. An important approach for reverse genetic studies is FST analysis. Sequences flanking T-DNA are determined for each transgenic line thereby leading to the development of a database of T-DNA tagged genes that can be searched electronically.

Although a large number of rice mutants tagged by T-DNA have been produced worldwide, the number (88,000) of gene activation lines remains small relative to the number (288,000) of gene knockout lines (Guiderdoni *et al.*, 2006). As described above, gene activation lines have advantages not offered by gene knockout lines, we set out and significantly expanding the collection of gene activation lines currently available to researchers. The T-DNA was also specially designed for trapping of genes or promoters that are active in special tissues, growth stages or under certain growth conditions. Currently, we have generated 60,000 fertile independent transgenic rice lines tagged with T-DNA and analyzed their FSTs. A total of 15,000 FSTs have been obtained from 30,000 transgenic lines, mapped to the genome of rice

cultivar Tainung 67, and analyzed (Hsing *et al.*, 2006). This resource provides a valuable tool for the determination of rice gene functions on a genomic scale, and a searchable FST database is available at the Taiwan Rice Insertional Mutant (TRIM) website (http://trim.sinica.edu.tw/).

MATERIALS AND METHODS

Generation of gene knockout or activation tagged rice mutant lines

Rice cultivar (*Oryza sativa* cv. Tainung 67) have been transformed with the multi-functional plasmid pTag8 (Fig. 1) *via Agrobacterium*-mediated transformation. T0 transgenic rice plants regenerated from tissue culture have been shipped to Taiwan Agriculture Research Institute (TARI), with an average rate of approximately 1000-1500 lines per month. Insertion of T-DNA into different genic regions lead to either gene knockout or gene knockout (Fig. 2). The pipeline for generation of T-DNA tagged rice mutant library and its application in basic research and crop improvement is shown in Fig. 3. The consortium that integrates the multidiscipline expertise and resources for team efforts is shown in Fig. 4. The examples of some mutants are shown in Fig. 5. The standard procedures for study a mutant tagged by T-DNA is shown in Fig. 6.

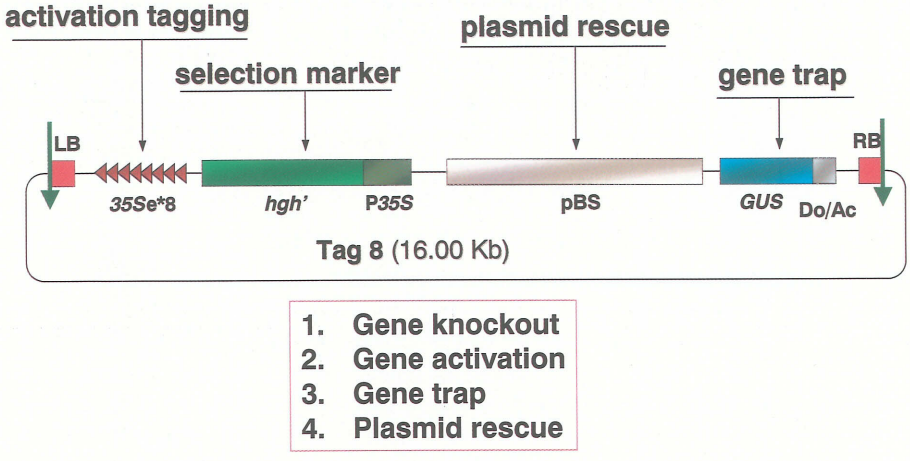

Fig. 1. Multiple-functional T-DNA for Gene Knockout/Activation Tagging

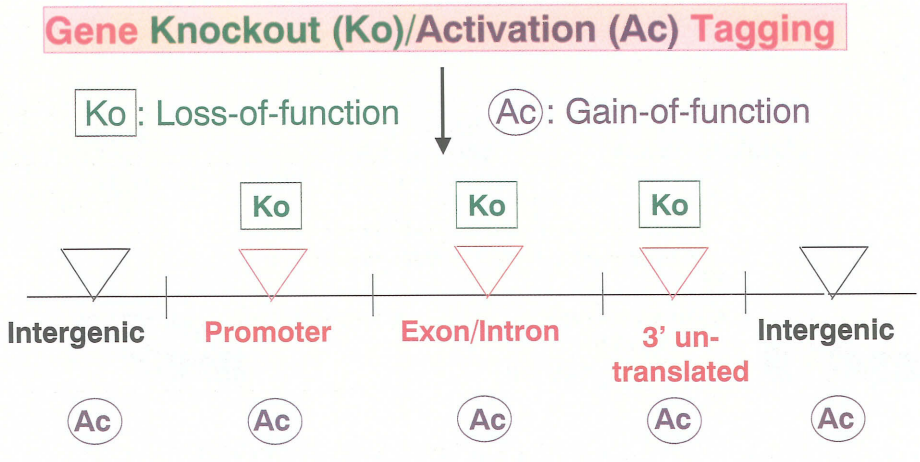

Fig. 2 T-DNA insertional mutagenesis leads to gene knockout or activation

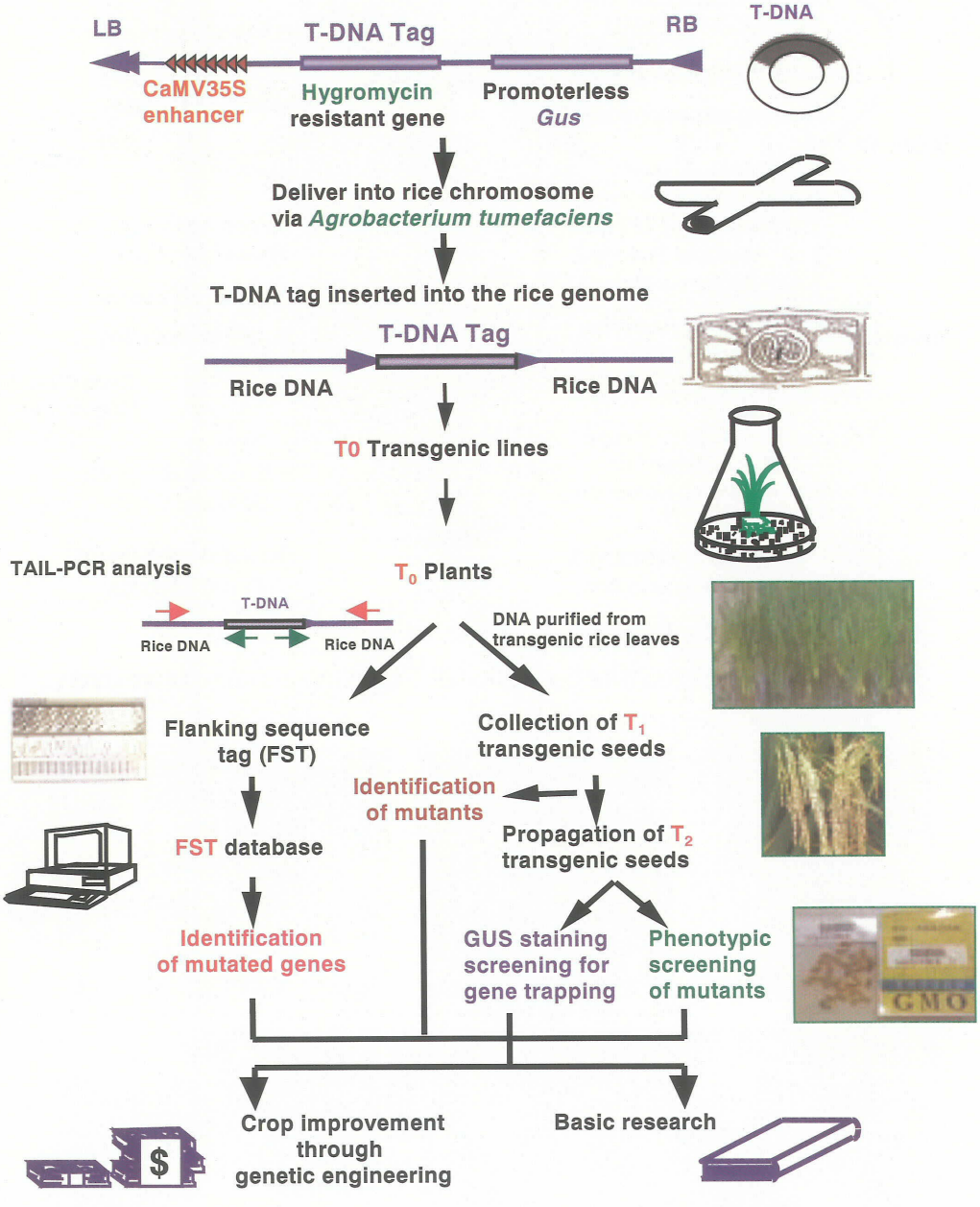

Fig.3 Generation and Utility of Rice Gene Knockout/Activation Library

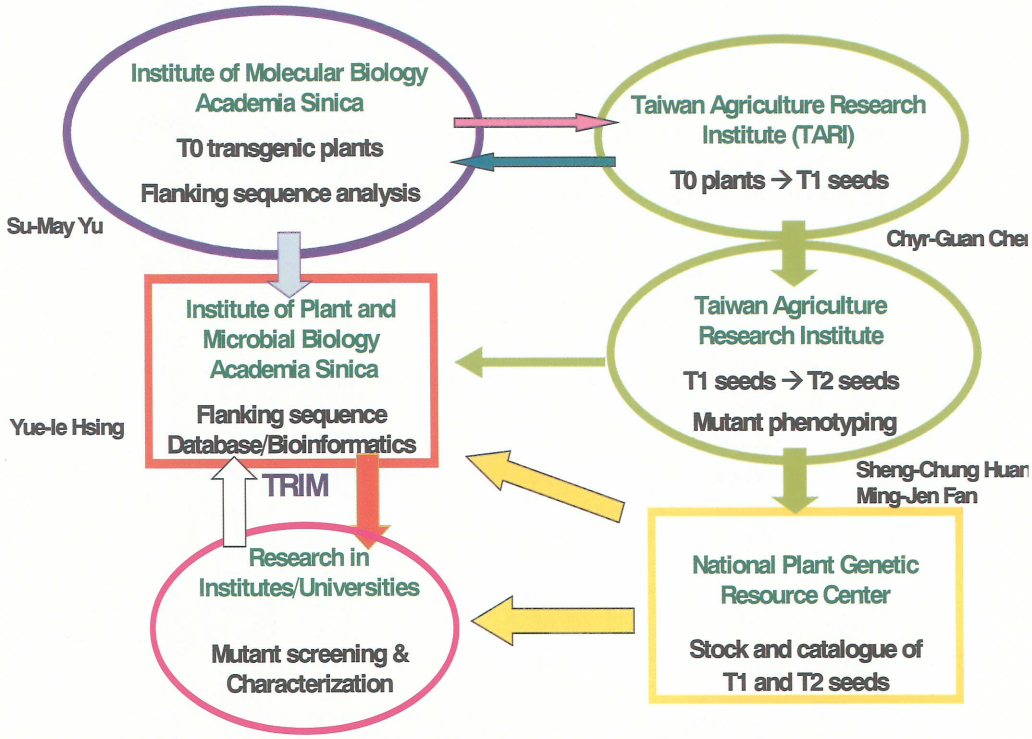

Fig. 4. Consortium for generation of T-DNA tagged rice mutant library.

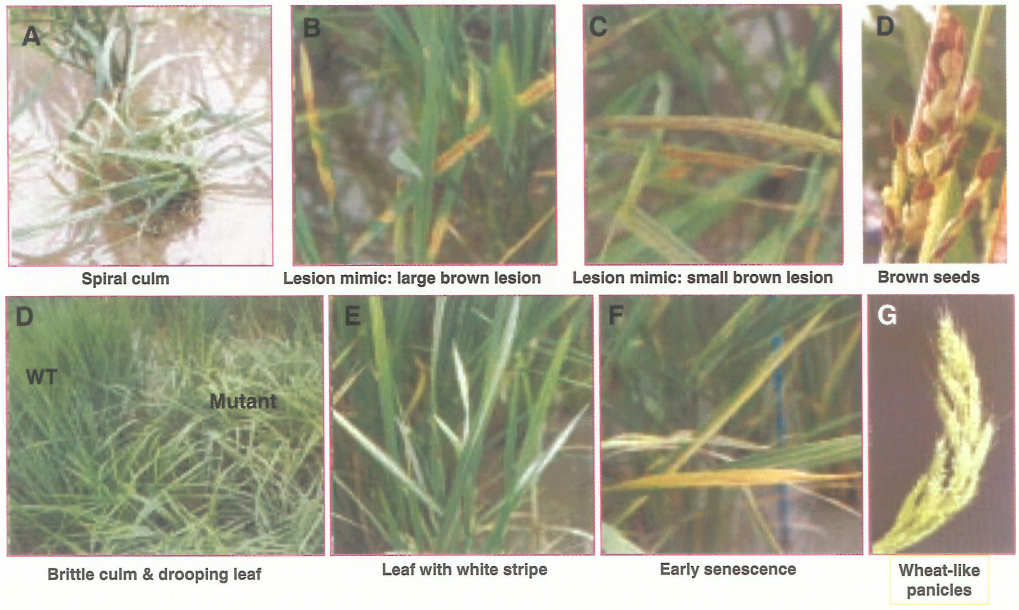

Fig. 5. Examples of rice mutants selected for study.

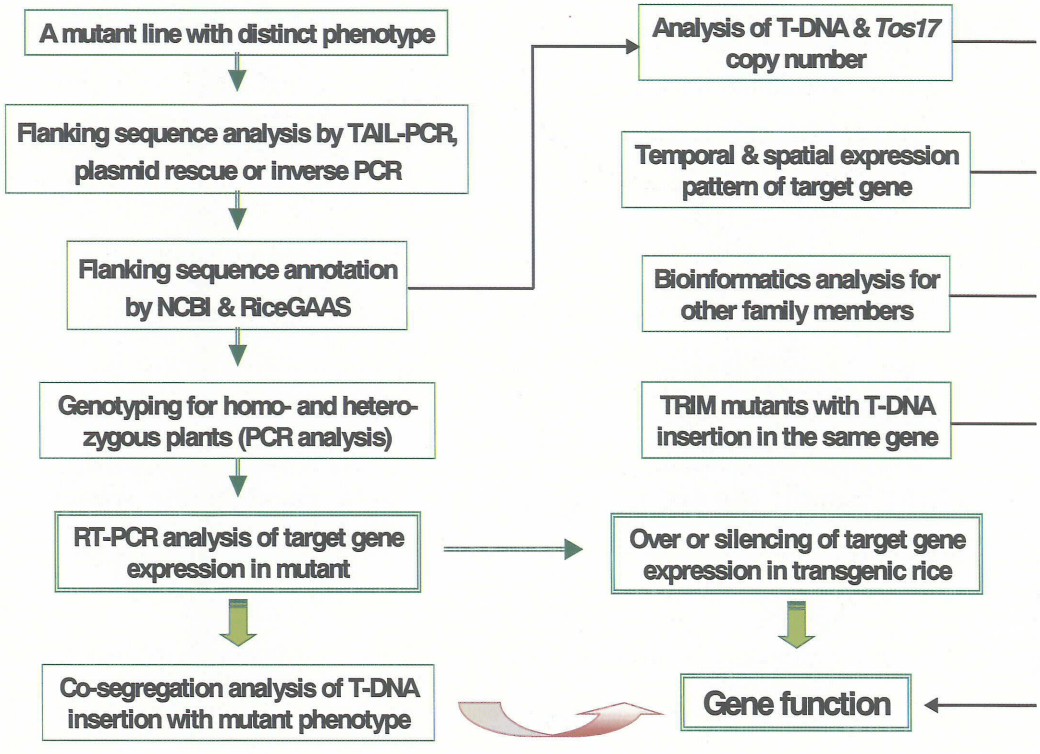
A mutant line with distinct phenotype
Flanking sequence analysis by TAIL-PCR, plasmid rescue or inverse PCR
Flanking sequence annotation by NCBI & RiceGAAS
Genotyping for homo- and hetero-zygous plants (PCR analysis)
RT-PCR analysis of target gene expression in mutant
Co-segregation analysis of T-DNA insertion with mutant phenotype
Analysis of T-DNA & Tos17 copy number
Temporal & spatial expression pattern of target gene
Bioinformatics analysis for other family members
TRIM mutants with T-DNA insertion in the same gene
Over or silencing of target gene expression in transgenic rice
Gene function

Fig. 6. Standard procedure for studying T-DNA tagged rice mutants.

RESULTS

Several labs joined the research team effort for screening and characterization of rice mutants

Currently, about two dozens of labs have obtained T2 seeds of the TRIM mutant library from the National Plant Genetic Resource Center and are screening and studying mutants with altered responses to biotic or abiotic stresses, or with altered growth and development phenotypes. Among these labs, 12 of them have applied and/or are currently carried out National Agricultural Biotechnology Program

projects. Several putative mutants identified through forward genetics approaches, including (a) insensitive or hypersensitive to salt and water stresses; (b) insensitive or hypersensitive to sugar or hormones; (c) altered growth rate, seed size and color, height, nutrient uptake, flowering time and yield; and (d) resistant to disease and insect, have been identified. Many mutants with essential genes, mainly signaling and transcription factors, being tagged by T-DNA, through reverse genetics approaches, have also being selected. These mutants and mutated genes are being characterized. The examples of some mutants are shown in Fig. 5.

The TRIM mutant library is a precious research resource for science and agriculture biotechnology

The TRIM mutant resources offer excellent tools for both basic and applied research of rice and other cereals and are available to the science community in Taiwan. The TRIM database facilitates identification of genes that are functional in biochemical pathways, physiological processes, or growth and development of cereal crops. Detailed characterization of the gene knockout/activation mutants would validate functions of these genes. Many of these genes could be valuable for crop improvement, e.g., to enhance biotic and abiotic stress tolerance, productivity, and quality of cereal crops, through genetic engineering approaches. Accomplishment of this project has a major impact on the development of agriculture biotechnology in Taiwan as well as promotion of the Taiwanese rice functional genomics projects in the worldwide arena.

CONCLUSIONS

The TRIM mutant library provides an opportunity for international collaborations

The TRIM project is launched in 2002, several years behind other labs at the beginning. However, the project has been progressed at a good pace. Currently, TRIM mutant resources ranked No. 3 among the four largest T-DNA insertion libraries (China, Korea, Taiwan and France) in the world. However, in terms of the user-friendly utility, information provided, and number of available T2 seeds, the TRIM mutant resource is ranking close to the top. Promotion has been conducted to encourage the plant science community in Taiwan to make the best utilization of this library for rice functional genomics research. Many labs are expected to be productive in next several years. Due to the distinguished achievement in establishing the TRIM mutant resources, Dr. Yu was recently invited to contribute two articles, together with many internationally well-known scientists, for the book *Rice Functional Genomics* to be published by Springer. Establishment of the TRIM resources has set up a milestone for rice functional genomics research for the Taiwanese science community as well as opportunity for promoting worldwide collaborations for cereal crop improvement.

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